Effects of *Stachys Byzantina* C. Koch Aerial Parts Aqueous Extract on Morphine Dependence and Tolerance in Mice

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ABSTRACT

Due to interaction of Lamiaceae plants with opioid system, we decided to evaluate the effect of another plant of this family, *Stachys byzantina* C. Koch., on withdrawal syndrome and tolerance to antinociceptive action of morphine. Dependence was induced using subcutaneous (S.C) injection of morphine daily for 3 days. On day 4, morphine was injected 0.5 h after the intraperitoneal (i.p) injection of extract or clonidine (0.3 mg/kg, as positive control). Naloxone was injected (5 mg/kg, S.C) 2h after the final dose of morphine. The number of episodes of jumping during 30 minutes after injection of naloxone was considered as the intensity of the withdrawal syndrome. Tolerance to morphine was induced by twice-daily injection of morphine (20 mg/kg, S.C.) for 4 days. The antinociceptive response to morphine (10 mg/kg, S.C) was determined on day 1 and 6 using the tail flick test. The effect of different doses of aqueous extract of *S. byzantina* (0.04-0.4 g/kg), twice-daily, was determined on tolerance to the antinociceptive action of morphine. The aqueous extract was not injected on day 6. The locomotor activity of the aqueous extract was evaluated using the open field activity test in mice. The aqueous extract decreased the jumping episode significantly. In addition, the aqueous extract of *S. byzantina* attenuated the development to tolerance to the antinociceptive action of morphine in mice. None of doses reduced the locomotion activity in mice. We can conclude that the aqueous extract of *S. byzantina* reduced morphine withdrawal syndrome and tolerance.

Key words: *Stachys byzantina*, morphine dependence, tolerance to morphine, withdrawal syndrome, opioid system.

INTRODUCTION

Stachys is a genus of about 200 species of flowering plants in the family Lamiaceae, found in Mediterranean area and Iran (as Alborz and Kandovan) (1). *Stachys byzantina* C. Koch (Lamb's Ear) is a species of *Stachys*. It has been found out in recent research that *S. byzantina* has anti-microbial (2-3) and antioxidant effects (4-5).

The essential oils of the dried flowering aerial parts of *S. byzantina* were isolated by hydrodistillation and analysed by means of GC and GC-MS. The major components of *S. byzantina* oil were piperitenone (9.9%), 6, 10, 14-trimethyl pentadecan-2-one (6.4%), and n-tricosane (6.4%) (6). In another study, both hydrodistilled and steam distilled essential oils of the aerial parts of *S. byzantina* were rich in sesquiterpenes such as α-copaene (16.6% and 10.4%), spathulenol (16.1% and 18.5%) and β-caryophyllene (14.3% and 13.5%), respectively (7).

The potential to reduce morphine withdrawal signs in animals has been reported for some Lamiaceae plants and others (8) such as *Rosmarinus officinalis* (9-10), *Nepeta glomerulosa* (11), *Salvia lerifolia* (12), *Berberis vulgaris* (13), *Ferula gummosa* (14), *Zhumeria majdae* (15) and *Marrubium vulgare* (16). Therefore, this study was initiated to investigate the effects of aerial parts of aqueous extracts of *S. byzantina* C. Koch as a Lmiaceae plant on morphine dependence and tolerance in mice.

MATERIAL AND METHODS

Animals

Male albino mice (25-30 g) were housed at 22 ± 2ºC under a 12h light /12 h dark cycle and with access to food and water *ad libitum*. The experiments were performed during the light phase of the cycle. The animal groups were acclimated to the laboratory for at least 2 h before testing and were used once through the experiments were carried out in accordance with Mashhad University of Medical Sciences, Ethical Committee Acts.

Drugs

The following drugs were used: morphine sulphate (Daru Pakhsh, Iran), clonidine (Daru pakhsh, Iran), naloxone hydrochloride (Tolidaru, Iran). The drugs were dissolved in saline and were injected intraperitoneally (I.P.) in a volume of 10 ml/kg, except morphine which was administered subcutaneously (S.C.). The control groups received saline.

Plant material

The plant material (aerial parts of *S. byzantina* C. Koch) was collected in Bojnourd suburbs (Darkesh and

Haver) 1590 m height, I. R. Iran, a voucher specimen was deposited at the Dr. Zargari Herbarium, Department of Pharmacognosy, School of Pharmacy, Mashhad (152-1902-1).

Preparation of plant extract

The powder of aerial parts was extracted using the aqueous decoction. In the decoction method, 100 g powder was added to 1 L of boiling water for 15 min and then filtered through a cloth. The extract was then concentrated under reduced pressure (in a rotavaporator at 40ºC) to the desired volume. The extracts were dissolved in normal saline.

Morphine dependence

Dependence was induced by subcutaneously injection of morphine (50, 50 and 70 mg/kg) three times a day (0.8.00, 11.00 and 14.00 h) for three days. The higher dose at the daily 14 P.M. injection was aimed at minimizing any overnight withdrawal on day 4 they received a last dose of morphine (50 mg/kg) [17].

Morphine withdrawal syndrome

Opioid receptor antagonist, naloxone (5 mg/kg), was used inducing withdrawal syndrome after morphine physical dependence. Mice were placed individually in glass cube boxes (28 cm diameter, 50 cm height). The number of jumps (as a main sign of withdrawal syndrome in mice) was recorded over a 20 min., beginning immediately after the injection of naloxone, 2 h after the final administration of morphine [17].

The extracts, in doses 0.04, 0.16, 0.28, 0.4 g/kg, and clonidine 0.3 mg/kg injected intraperitoneally 1.5 h before naloxone treatment in each group in order to evaluate morphine dependence.

Measurement of locomotor activity

To evaluate the effect of drugs on locomotor activity, all of the animal in withdrawal test, were subjected to open field test before injection of naloxone. The open-field (100 cm width × 100 cm length × 40 cm height) was divided into a 5 × 5 grid of equally sized squares using black tape. Each mouse was gently placed in the center of the box and activity was scored as a line crossing when a mouse removed all four paws from one square and entered another. Line crossings in the squares were counted for 10 min.

The parameters evaluated were the total number of square crossed, the number of outer squares (those adjacent to the walls) crossed, and the number of inner square crossed; the three measures were referred the total, peripheral, and central locomotion, respectively (18).

Induction and evaluation of morphine tolerance

A 4-day dosing regimen was used for the induction of morphine tolerance (19). Morphine was injected to each mouse at dose 20 mg/kg (S.C.) twice-daily (9:00 and 16:30) for 4 days (days 2 to 5). On day 1 and 6, mice were tested for antinociceptive activity (tail-flick test) 30 min following morphine administration (10 mg/kg; S.C.) and the result compared to determine the intensity of tolerance to antinociceptive effect of morphine.

To evaluate the effect of extracts on development of morphine tolerance, on days 2 to 5, the extract (0.04, 0.16 and 0.28 mg/kg, I.P.) was injected simultaneously with morphine and mice were tested for antinociceptive activity 30 min following morphine administration (10 mg/kg; S.C.) on day 6.

Antinociceptive activity was assessed using the radiant heat tail-flick test (Sparko, Co, Iran). Thirty min after drug injections, the tail-flick latencies were measured. A cut off time of 10 s was used to prevent tissue damage.

Phytochemical screening

The methanol extract was screened for alkaloids, flavonoids, tannins and saponins (20-21).

Statistical analysis

The results are presented as mean ± SEM. Statistical significance of the differences between the groups was detected by ANOVA, followed by Tukey-Kramer comparison test. P-values of less than 0.05 were considered as indicative of significance.

RESULTS

Phytochemical screening

Preliminary phytochemical screening of the *S.* byzantina indicated the presence of tannins and flavonoids.

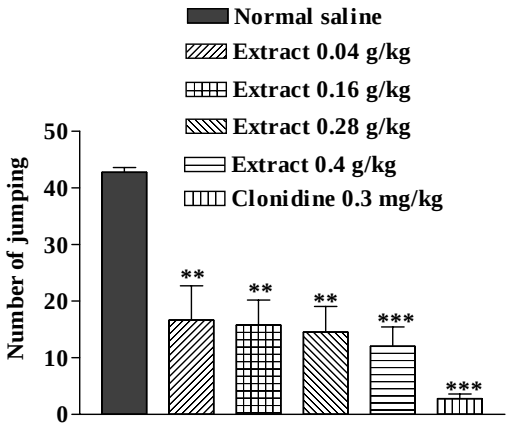

Figure 1. Effect of aqueous extract of *Stachys byzantina* aerial parts on morphine withdrawal syndrome in 8 male mice. 10 mg/kg morphine S.C. was injected, 3 times a day, during 3 days and one dose injected on day 4. Withdrawal syndrome was induced by the I.P. injection of naloxone (5 mg/kg) 90 min after the I.P. treatments of extracts, clonidine or normal saline; Data was reported as Mean + SEM. **P<0.01, ***P<0.001 *vs* saline, Tukey-Kramer test.

Effect of extract on morphine withdrawal

The administration of extracts (0.04-0.4 g/kg) reduced the number of jumping episodes significantly which this effect decreased with increasing the doses. However, this effect was not dose dependent (Figure 1).

Effect of extract on locomotor activity

Clonidine decreased open field test locomotion factors, central, peripheral and total locomotion activities. The extract did not show any effect on open field factors (Figure 2).

Effect of extract on tolerance

Even at the high dose (0.4 g/kg), the extract did not show antinociceptive activity alone. However, the extracts reduced the tolerance to morphine antinocicpetive activity on 6 th day (Figure 3).

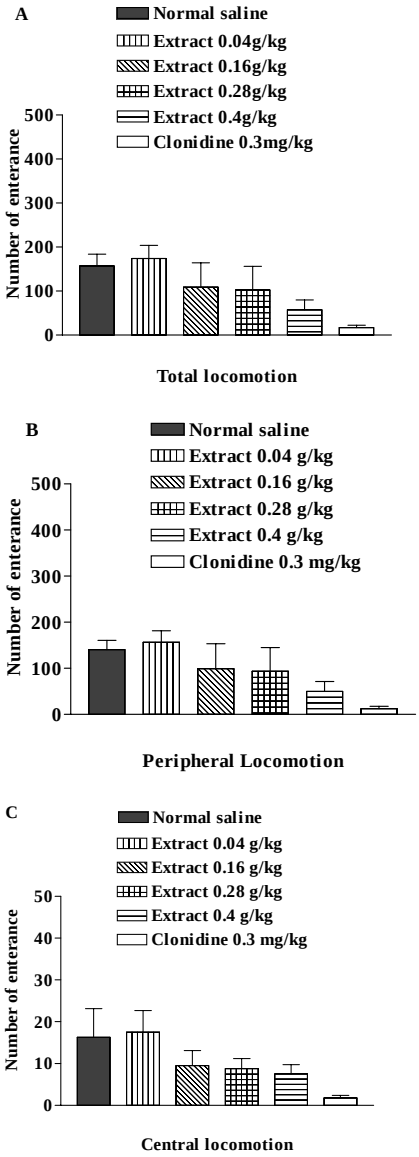

Figure 2. Effect of aqueous extract of *Stachys byzantina* aerial parts on open field test factors: total (A), peripheral (B) and central locomotion(C) in 8 male mice during 10 min. The experiment was done 60 min after extracts injections. Data were reported as Mean + SEM, **P<0.01, ***P<0.001 *vs* saline, Tukey-Kramer test.

DISCUSSION

The results of the present investigation demonstrate that the aqueous extract of *S. byzantina* aerial parts decreased the morphine withdrawal syndrome dose independently. It also attenuates the tolerance to antinociceptive effect of morphine.

Flavonoides have a major role in controlling withdrawal syndrome and morphine tolerance. Quercetin reversed morphine tolerance and dependence via its ability to suppress nitric oxide synthase (NOS) activity (22).

Gossypin, a flavonoid, pre-treatment significantly attenuated the acute tolerance development to morphine induce antinociceptive response (23).

Quercetin, flavone, catechin and chrysin were capable of blocking naloxone-induced contracture of the acute morphine-dependent guinea-pig ileum in a concentration-dependent fashion (24). Phytochemical experiments have shown the present of flavonoid and tanin in the plant. Thus, it is possible a flavonoid component of extract involves in reduction of tolerance or withdrawal syndrome.

Our result showed that the extract did not decrease locomotion activity. Therefore, depressant effects did not affect on reducing the jumping episodes.

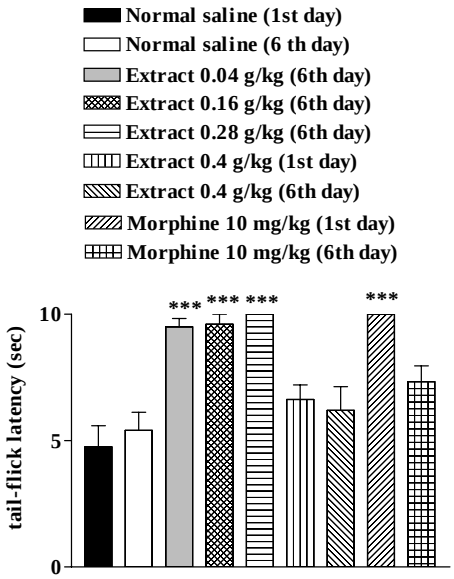

Figure 3. Effect of aqueous extract of *Stachys byzantina* aerial parts on morphine tolerance in 8 male mice. The animals received morphine (20 mg/kg twice-daily for 4 days) for induction of morphine tolerance. On the next day, animals received the extract (I.P.) or saline and morphine (10 mg/kg) and tail flick test were carried out to evaluate the effect of extracts on expression of morphine tolerance. Data are means + S.E.M. ***P<0.001 *vs* control, Tukey-Kramer test.

It is concluded that *S. byzantina* extract could decrease withdrawal syndrome and tolerance to morphine.

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